Molecular identification of the uptake carriers for organic cations in liver

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POLYSPECIFIC ORGANIC ANION TRANSPORTING POLYPEPTIDES MEDIATE HEPATIC UPTAKE OF AMPHIPATHIC TYPE II ORGANIC CATIONS

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ABSTRACT

Hepatic uptake of albumin-bound amphipathic organic cations has been suggested to be mediated by multispecific bile salt and organic anion transport systems. Therefore, we investigated whether the recently cloned rat organic anion transporting polypeptides 1 and 2 as well as the human organic anion transporting polypeptide might be involved in the hepatocellular uptake of bulky type II organic cations. In cRNA-injected *Xenopus laevis* oocytes, all three carriers mediated uptake of the known type II model compounds *N*-(4,4-azo-*n*-pentyl)-21-deoxy-ajmalinium and rocuronium, whereas the newly synthesized type II model compounds *N*-methyl-quinine and *N*-methyl-quinidine were transported only by the human organic anion transporting polypeptide. This carrier-mediated uptake of *N*-methyl-quinine and *N*-methyl-quinidine was sodium-independent and saturable with apparent K_m-values of ~ 5 µM and ~ 26 µM, respectively. In contrast to bulky type II organic cations, more hydrophilic type I organic cations such as tributylmethylammonium and choline were not transported by any of the organic anion transporting polypeptides. These findings demonstrate that organic anion transporting polypeptides can also mediate hepatocellular uptake of type II organic cations, whereas uptake of small and more water-soluble type I cations is mediated by different transport systems such as the organic cation transporters.

INTRODUCTION

The liver plays an essential role in the elimination of cationic drugs. The category of cationic drugs includes a large variety of compounds containing tertiary or quaternary amine groups or other positively charged functional groups. Quaternary amines are permanently positively charged, whereas tertiary amines acquire a charge by protonation depending on their pK_a value and pH of the medium (Meijer et al., 1997). Based on functional studies in the isolated perfused rat liver and with rat hepatocytes, organic cations have been classified into type I and type II compounds (Steen et al., 1992; Oude Elferink et al., 1995). Type I cations are small and relatively hydrophilic quaternary ammonium compounds, such as tetraethylammonium or molecules in which the cationic amine group is at some distance from the aromatic ring structure (e.g., procainamide ethobromide). Their hepatocellular uptake can be inhibited by choline and type II cations but not by bile salts and cardiac glycosides. Type II organic cations contain a positively charged group situated within or close to large aromatic ring structures (e.g., rocuronium). Their hepatic uptake is not affected by a large excess of choline and type I cations, but can be strongly inhibited by cardiac glycosides and bile salts such as digoxin and k-strophantoside indicating the involvement of one (or several) multispecific and charge-independent sinusoidal uptake system or systems (Steen et al., 1992).

Several organic cation transporters (OCTs) have recently been cloned from a variety of tissues and species, including rat and human liver (Grundemann et al., 1994; Zhang et al., 1997). Rat and human OCT1 are expressed at the basolateral plasma membrane of hepatocytes, where they mediate electrogenic uptake of typical type I organic cations such as tetraethylammonium, *N*-methyl-4-pyridinium, and choline (Koepsell, 1998). In contrast, bulky type II organic cations are not transported by the members of the OCT family of membrane transporters (Nagel et al., 1997),

although they can inhibit OCT-mediated type I cation transport (Zhang et al., 1997; Koepsell, 1998). However, the permanently charged type II organic cation *N*-(4,4-azo-*n*-pentyl)-21-deoxyajmalinium (APDA) has been suggested to be a substrate of the so-called organic anion transporting polypeptides (Oatps; Bossuyt et al., 1996a,b). These polyspecific transporters mediate sodium-independent hepatocellular uptake of a wide variety of amphiphilic albumin-bound compounds, including bile salts, organic anionic dyes (e.g., sulfobromophthalein), steroid-conjugates, cardiac glycosides (e.g., ouabain, digoxin) and peptidomimetic drugs (Meier et al., 1997). Because bile salts and cardiac glycosides have been shown to inhibit sodium-independent uptake of type II organic cations into rat and human hepatocytes (Steen et al., 1992), we tested the hypothesis that members of the *Oatp* gene family of membrane transporters are involved in the hepatic uptake of type II organic cations. The results support the general conclusion that bulky type II organic cations are taken up into hepatocytes by Oatps, whereas cellular uptake of small and more water-soluble type I cations is mediated by other systems such as the OCTs, which belong to a distinct family of amphiphilic solute transporter (Schomig et al., 1998).

EXPERIMENTAL PROCEDURES

Materials

[^{3}H]Estrone-3-sulfate (53 Ci/mmol), [^{3}H]digoxin (15 Ci/mmol), and [^{3}H]dehydroepiandrosterone sulfate (16 Ci/mmol) were obtained from Du Pont-New England Nuclear (Boston, MA). [*methyl*-^{3}H]Choline chloride (83 Ci/mmol) was purchased from Amersham Pharmacia Biotech (Buckinghamshire, England). [^{14}C]Rocuronium (54 mCi/mmol) and unlabeled rocuronium were kind gifts of Organon International BV (Oss, The Netherlands). APDA (1.2 Ci/mmol), *N*-(4,4-azo-*n*-pentyl)-quinuclidine (APQ; 2.5 Ci/mmol), and unlabeled APQ were synthesized as described previously (Müller et al., 1994). [^{3}H]Tributylmethylammonium (TBuMA; 85 Ci/mmol) was synthesized according to Neef et al. (1984). Unlabeled TBuMA was obtained from Fluka (Buchs, Switzerland). The new type II cation model compounds [^{3}H]*N*-methyl-quinine (85 Ci/mmol) and [^{3}H]*N*-methyl-quinidine (85 Ci/mmol) were synthesized through the methylation of quinine and quinidine, respectively, with [^{3}H]methyl iodide (Amersham Pharmacia Biotech) according to the procedures described for the synthesis of [^{3}H]TBuMA (Neef et al., 1984). Unlabeled N-methyl-quinine and N-methyl-quinidine were also obtained through the methylation of quinine and quinidine, respectively. The molecular weight of the new compounds was assessed by mass spectroscopy, confirming the methylation of the quinuclidine nitrogen. [^{3}H]Azidoprocainamide methoiodide (APM; 85 Ci/mmol) and unlabeled APM were synthesized as described (Mol et al., 1992). Radiochemical purity of the substrates not available commercially was determined by thin-layer chromatography and exceeded 99%. All other chemicals were of analytical grade and readily available from commercial sources.

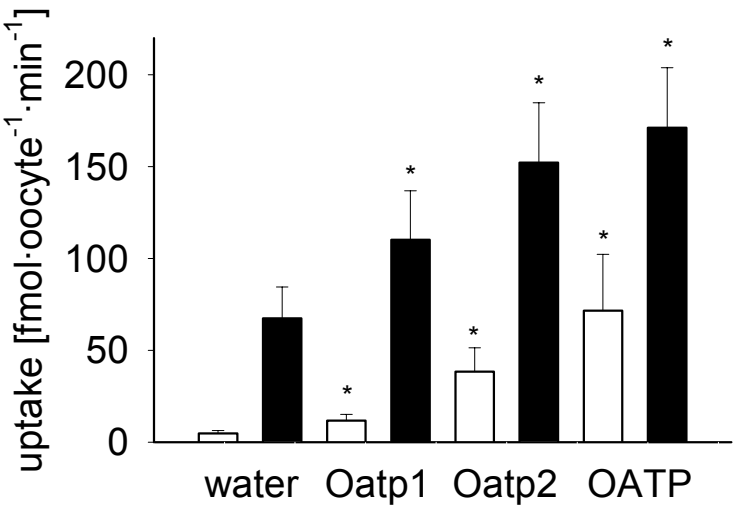

Figure 1. Comparison of Oatp1-, Oatp2-, and OATP-mediated uptake of APDA and rocuronium by *X. laevis* oocytes. *X.laevis* oocytes were injected with 5 ng of Oatp1-cRNA, 5 ng of Oatp2-cRNA, or 2.5 ng of OATP-cRNA. After 3 days in culture, uptakes of [^{3}H]APDA (2 μM; open columns) and [^{14}C]rocuronium (14 μM; filled columns) were measured at 30 min in PBS (see *Experimental Procedures*). Data represent the mean ± S.D. of 14 to 15 oocyte uptake measurements. *Significantly different from water injected control oocytes (Mann-Whitney *U* test, $p < 0.001$).

Uptake studies in *Xenopus laevis* oocytes

In vitro synthesis of rat organic anion transporting polypeptide 1 (Oatp1)-cRNA, rat organic anion transporting polypeptide 2 (Oatp2)-cRNA, and human organic anion transporting polypeptide (OATP) (in accordance with international guidelines, see http://ratmap.gen.gu.se/ratmap/WWWNomen/Nomen.html and http://ash.gene.ucl.ac.uk/nomenclature/)-cRNA was performed as described previously (Kullak-Ublick et al., 1994, 1995; Noé et al., 1997). *X. laevis* oocytes were prepared (Hagenbuch et al., 1996) and cultured overnight at 18°C. Healthy oocytes were microinjected with 5 ng of Oatp1-cRNA, 5 ng of Oatp2-cRNA, or 2.5 ng of OATP-cRNA and cultured for 3 days in a medium containing 88 mM NaCl, 2.4 mM $NaHCO_3$, 1 mM KCl, 0.3 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 0.05 mg/ml gentamycin, and 15 mM HEPES (pH 7.6). Unless stated otherwise (see the legend to Fig. 4), all tracer uptake studies were performed in PBS (137 mM NaCl, 2.7 mM KCl, 10.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4). The oocytes were prewashed in the uptake medium and then incubated at 25°C in 100 μl of the uptake medium containing the indicated substrate concentrations. Water-injected oocytes were used as controls for unspecific uptake of the substrate. After the indicated time intervals, uptake was stopped by addition of 6 ml ice-cold uptake medium. The oocytes were washed twice with 6 ml ice-cold uptake medium. Subsequently, each oocyte was dissolved in 0.5 ml of 10% SDS and 5 ml of scintillation fluid (Ultima Gold, Canberra Packard, Zürich, Switzerland), and the oocyte-associated radioactivity was determined in a Tri-Carb 2200 CA liquid scintillation analyzer (Canberra Packard). Determination of kinetic uptake parameters was performed with a nonlinear curve-fitting program (Systat 6.0.1, SPSS Inc., Chicago, IL) using a simple Michaelis-Menten model ($v = V_{max} \cdot [S]/(K_m+[S])$).

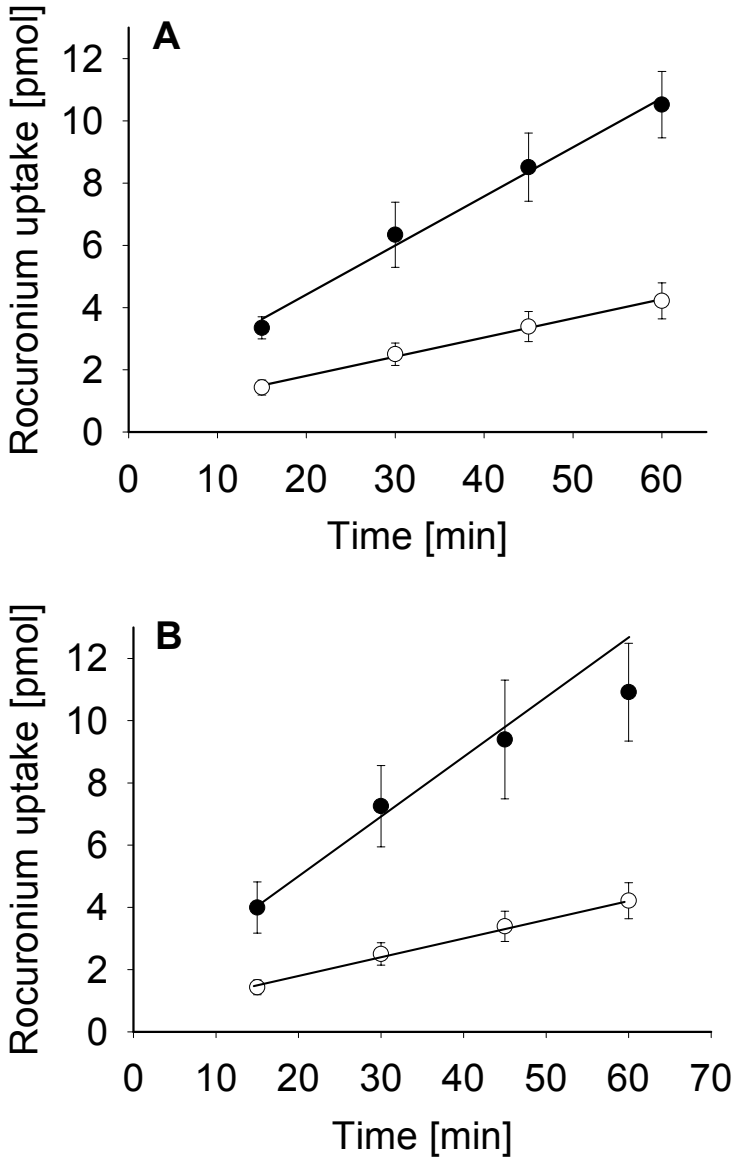

Figure 2. Time course of rocuronium uptake in Oatp2-cRNA and OATP-cRNA injected *X. laevis* oocytes. *X.laevis* oocytes were injected with 5 ng of Oatp2-cRNA or 2.5 ng OATP-cRNA. After 3 days in culture, the time courses for 13 μM [^{14}C]rocuronium were measured for Oatp2-cRNA- (A) and OATP-cRNA- (B) injected oocytes in PBS (see *Experimental Procedures*). Filled circles, Oatp2-cRNA- (A) and OATP-cRNA- (B) injected oocytes, respectively. Open circles, water-injected control oocytes. Data are expressed as mean ± S.D. of 13 to 15 oocyte uptake measurements.

Statistical analysis

Uptake results are given as means ± S.D. Statistical significance of transport differences between the various oocyte groups was determined with the Mann-Whitney *U* test (Systat 6.0.1, SPSS Inc., Chicago, IL).

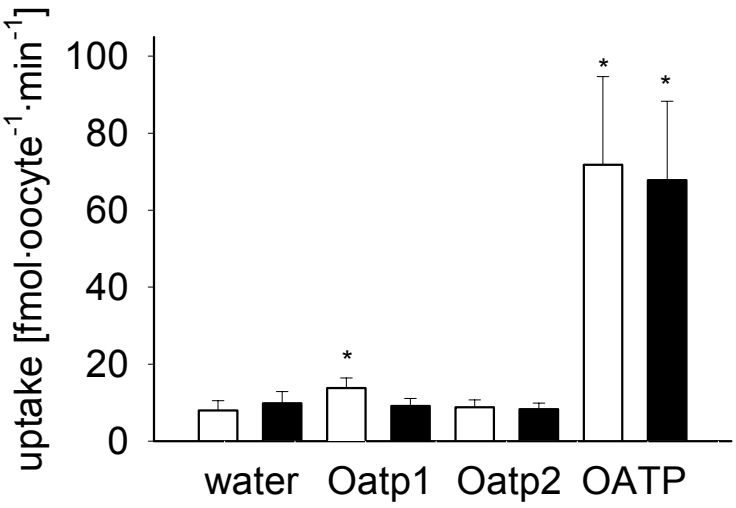

Figure 3. Comparison of Oatp1-cRNA-, Oatp2-cRNA, and OATP-cRNA-mediated uptake of *N*-methyl-quinine and *N*-methyl-quinidine by *X. laevis* oocytes. *X.laevis* oocytes were injected with 5 ng of Oatp1-cRNA, 5 ng of Oatp2-cRNA, or 2.5 ng of OATP-cRNA. After 3 days in culture, uptakes of [^{3}H]-N-methyl-quinine (2 μM; open columns) and [^{3}H]*N*-methyl-quinidine (2 μM; filled columns) were measured at 30 min in PBS (see *Experimental Procedures*). Data represent the mean ± S.D. of 13 to 15 oocyte uptake measurements. *Significantly different from water injected control oocytes (Mann-Whitney *U* test, $p < 0.001$).

RESULTS

To determine the involvement of the cloned Oatps in organic cation transport, uptakes of the known type II cations APDA and rocuronium (Steen et al., 1992; Meijer et al., 1997) were measured in Oatp1-cRNA-, Oatp2-cRNA-, and OATP-cRNA-injected *X. laevis* oocytes. In this manner, the injected amounts of cRNA were chosen on the basis of preliminary experiments showing maximal substrate uptake activities at 5.0 ng (Oatp1, Oatp2) and 2.5 ng (OATP), respectively. As illustrated in Fig. 1, all three carriers mediated significant uptake of both substrates. For APDA, these data confirm previous findings with Oatp1 and OATP (Bossuyt et al., 1996a,b). Furthermore, they demonstrate that Oatp2 stimulated APDA uptake by approximately 8-fold. All three members of the *Oatp* gene family of membrane transporters can mediate transport of the type II organic cation APDA; thereby, the strongest stimulation of uptake was found for OATP (15-fold), followed by Oatp2 (8-fold) and Oatp1 (2.4-fold). A similar pattern of uptake stimulation was found for rocuronium, although the differences between the cRNA- and the water-injected control oocytes did not exceed a 3-fold stimulation of rocuronium uptake for all three carriers tested.

In time-course experiments, the uptake of rocuronium by Oatp2 and OATP was measured during 60 minutes (Fig. 2). Oatp2-mediated rocuronium uptake increased linearly during 60 minutes whereas OATP-mediated rocuronium uptake was linear for 45 minutes. Because rocuronium exhibited increasing unspecific diffusion and/or binding to the water-injected control oocytes at high substrate concentrations, we were not able to demonstrate clear-cut saturability of Oatp2- and OATP-mediated rocuronium uptake (data not shown). Also, the kinetic constants for Oatp-mediated APDA transport could not be determined because no unlabeled

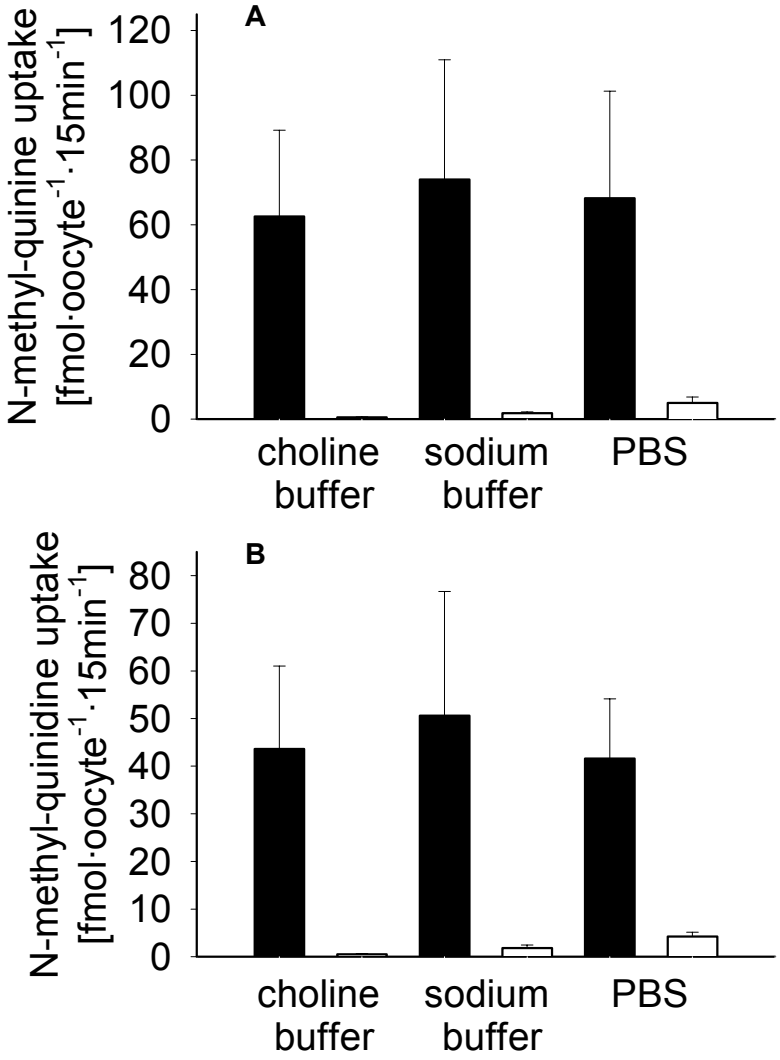

Figure 4. Comparison of OATP-cRNA-mediated uptakes of *N*-methyl-quinine and *N*-methyl-quinidine by *X. laevis* oocytes in the presence and absence of extracellular sodium. *X.laevis* oocytes were injected with 2.5 ng of OATP-cRNA. After 3 days in culture, uptake of [^{3}H]*N*-methyl-quinine (0.1 μM) and [^{3}H]*N*-methyl-quinidine (0.1 μM) were measured at 15 min in choline buffer (100 mM choline chloride, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM Tris-HEPES, pH 7.5), sodium buffer (100 mM NaCl instead of choline chloride) or PBS (see *Experimental Procedures*). Filled columns represent OATP-cRNA-injected oocytes, and open columns represent water-injected control oocytes. Data represent the mean ± S.D. of 11 to 15 oocyte uptake measurements.

APDA was available. Therefore, Oatp-mediated organic cation transport was further characterized with the new type II model compounds *N*-methyl-quinine and *N*-methyl-quinidine in Oatp1-cRNA, Oatp2-cRNA, and OATP-cRNA-injected oocytes. As illustrated in Fig. 3, OATP stimulated the uptake of *N*-methyl-quinine and *N*-methyl-quinidine by approximately 9- and 7-fold, respectively, compared with water-injected oocytes. The OATP-mediated uptakes of 10 μM *N*-methyl-quinidine and 10 μM taurocholate were mutually inhibited by 100 μM taurocholate and 100 μM *N*-methyl-quinidine to the extent of 37 and 96%, respectively (data not shown). Among the other carriers tested, only Oatp1 showed a significant, albeit low, uptake activity for *N*-methyl-quinine.

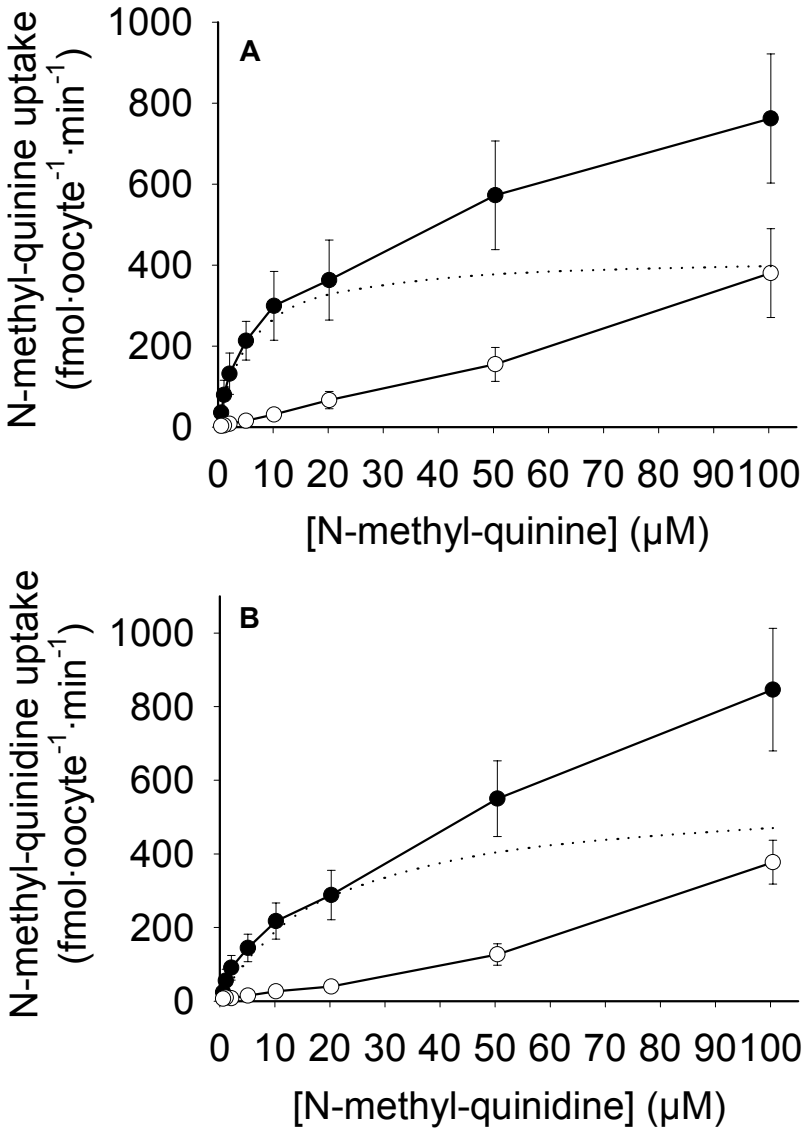

Figure 5. Saturation kinetics of *N*-methyl-quinine and *N*-methyl-quinidine uptake in OATP-cRNA-injected *X. laevis* oocytes. *X.laevis* oocytes were injected with 2.5 ng of OATP-cRNA. After 3 days in culture, uptakes of [^{3}H]*N*-methyl-quinine and [^{3}H]*N*-methyl-quinidine were measured at 15 min in PBS (see *Experimental Procedures*) containing increasing substrate concentrations. The dotted line represents uptake differences between OATP-cRNA (filled circles) and water-injected oocytes (open circles). Data are expressed as mean ± S.D. of 10 to 15 oocyte uptake measurements.

Uptake of *N*-methyl-quinine and *N*-methyl-quinidine by OATP increased linearly for at least 30 minutes (data not shown) and was independent of extracellular sodium as the uptake rates did not differ significantly between choline and sodium containing buffers (Fig. 4). This finding also shows that the type I organic cation choline does not interfere (i.e., no *cis*-inhibition) with the uptake of the tested new type II compounds. Because the uptake rates were not significantly different in PBS compared with choline and sodium buffers, the data indicate that PBS is also a suitable uptake medium for transport experiments in oocytes.

In all subsequent experiments designed to determine the kinetic parameters for OATP-mediated *N*-methyl-quinine and *N*-methyl-quinidine uptake, the oocytes

were incubated for 15 minutes in the presence of increasing substrate concentrations. OATP-mediated initial uptake rates were saturable and yielded apparent K_m-values of 5.1 ± 2.1 μM (mean ± S.E.) and 25.6 ± 4.1 μM for *N*-methyl-quinine and *N*-methyl-quinidine, respectively (Fig. 5). The V_{max}-values amounted to 329 ± 36 fmol/oocyte·min for *N*-methyl-quinine and 595 ± 36 fmol/oocyte·min for *N*-methyl-quinidine.

Finally, we also tested type I organic cations as possible substrates of the Oatps, although they have already been shown to be true substrates of members of the OCT family of organic cation transporters (Koepsell, 1998). As summarized in Table 1, none of the cationic type I substrates (i.e., TBuMA, APM, APQ, and choline) were significantly transported by the Oatps, indicating that type I organic cations are not substrates for the Oatps. In contrast, type II organic cations were again transported best by OATP, followed by Oatp2 and Oatp1 (see also Fig. 1). These results are validated by the 6- to 70-fold increase of Oatp1-, Oatp2-, and OATP-mediated uptake of the standard substrates estrone-3-sulfate, digoxin, and dehydroepiandrosterone sulfate, respectively (Table 1). Thus, although Oatp1 preferentially transports organic anions including steroid-conjugates, the OATP exhibits the highest transport activity for all type II cations tested with *N*-methyl-quinidine representing a possible OATP-specific substrate. These differences in the substrate specificity further support the assumption that the three Oatps are different gene products (Meier et al., 1997) and that the human orthologs of Oatp1 and Oatp2 remain to be identified.

DISCUSSION

The present study demonstrates that members of the *Oatp* gene family of membrane transporters can account for multispecific type II organic cation uptake into rat and human liver. While all three Oatps investigated can mediate transport of APDA and rocuronium to some extent (Table 1, Figs. 1 and 2), the newly synthesized model compounds *N*-methyl-quinine and *N*-methyl-quinidine were transported virtually only by the human OATP (Table 1, Fig. 3). In fact, *N*-methyl-quinidine might represent a specific substrate for OATP, while digoxin is a specific high affinity substrate for Oatp2 (Noé et al., 1997). The magnetic resonance liver imaging organic anion gadoxetate has been shown to be a specific substrate for Oatp1 (van Montfoort et al., 1999). These results further demonstrate that Oatp carriers exhibit partially overlapping and partially selective substrate specificities. In addition, the results support the concept that Oatps can mediate charge-independent substrate uptake into hepatocytes and thus play an important role in the disposition and hepatic clearance of a wide variety of albumin-bound amphipathic drugs and other xenobiotics (Meier et al., 1997).

Several members of the *Oatp* gene family of membrane transporters have been cloned and their transport properties are increasingly being elucidated (Meier et al., 1997). Oatp1 has been cloned from rat liver and is expressed at the basolateral membrane of hepatocytes (Bergwerk et al., 1996), the brush border of choroid plexus (Angeletti et al., 1997) and kidney proximal tubular cells (Bergwerk et al., 1996), as well as at the blood brain barrier endothelium (B. Gao and P.J.M., unpublished observations). It can function as an anion exchanger (Satlin et al., 1997; Li et al., 1998) and mediates transmembrane transport of a wide range of amphipathic

organic compounds (Meier et al., 1997). However, as further indicated by its low transport activity for type II organic cations (Table 1), the preferred substrates of Oatp1 appear to be albumin-bound organic anions such as bile salts, bromosulfophthalein, leukotriene C_4, steroid-conjugates, gadoxetate, and certain anionic peptides (Meier and Stieger, 1999). Oatp2 has been cloned from rat brain (Noé et al., 1997) and exhibits a 77% amino acid identity with Oatp1. It is expressed at the basolateral membrane of midzonal to perivenous hepatocytes (Reichel et al., 1999) and of choroid plexus epithelial cells, at the blood brain barrier endothelium (Gao et al., 1999) and in retinal cells (Abe et al., 1998). Its spectrum of transport substrates is partially overlapping with Oatp1, but Oatp2 does not transport certain organic anions such as BSP and gadoxetate (van Montfoort et al., 1999), has a preference for uncharged cardiac glycosides such as digoxin (Noé et al., 1997), and exhibits a higher transport activity for type II organic cations than Oatp1 (Table 1, Figs. 1 and 2). Oatp3 was recently cloned from rat retinal cells and is also expressed in the kidney (Abe et al., 1998) and intestine and in bile duct epithelial cells (Walters et al., 1998). Its spectrum of transport substrates has not yet been investigated extensively, but it includes taurocholate and the thyroide hormones T_3 and T_4 (Abe et al., 1998). OATP has been cloned from human liver, but it is also expressed in brain, kidney, lung, and testis (Kullak-Ublick et al., 1995). Its amino acid identities of 67% and 73% with Oatp1 and Oatp2, respectively, indicate that OATP does not represent the human ortholog of any of the two rat proteins. This conclusion is supported by the unique transport preference of OATP for *N*-methyl-quinine and *N*-methyl-quinidine (Fig. 3, Table 1). Nevertheless, although the human orthologs of Oatp1 and Oatp2 remain to be identified, our results clearly indicate that members of the *Oatp* gene family of membrane transporters play an important role in the hepatic clearance of amphipathic albumin-bound type II cationic drugs and thus complement the organic cation and anion transport properties of members of the 'major facilitator superfamily' (Marger and Saier, 1993) such as the OCTs and OATs (Schomig et al., 1998).

Numerous OCT isoforms were recently cloned from various species and organs, including rat and human liver (Grundemann et al., 1994; Okuda et al., 1996; Gorboulev et al., 1997; Zhang et al., 1997; Kekuda et al., 1998). The transport substrates of all OCTs include relatively water-soluble small organic cations such as tetraethylammonium, *N*-methyl-4-pyridinium and choline (Koepsell, 1998). These OCT substrates are not transported by Oatps as further indicated for the classical type I organic cations TBuMA, APM, APQ, and choline (Table 1). Similarly, small water-soluble organic anions are transported by members of the OAT subfamily, and none of these OAT-substrates are also transported by Oatps (Kullak-Ublick et al., 1994, 1995), with the possible exceptions of methotrexate (Saito et al., 1996; Sekine et al., 1997) and prostaglandin E_2 (Kanai et al., 1995; Sekine et al., 1998). Interestingly, the charge appears to discriminate whether a relatively hydrophilic organic compound is a substrate for OCTs or OATs, whereas the charge plays an obviously less discriminative role for transport of more hydrophobic molecules by Oatps. Although the exact structural characteristics of Oatp substrates are not yet known, it can be speculated so far that increased size and hydrophobicity (and consequently a larger degree of albumin binding) might be two important features for qualification as an Oatp substrate. This assumption is supported by a recent study indicating that increasing the alkyl chain length is associated with a decreased translocation of *n*-tetraalkylammonium compounds by the human OCT1 (Zhang et al., 1999). Hence, long chain *n*-tetraalkylammonium compounds might also be substrates of the human OATP. This speculation, as well as the assumption that in

principle Oatps should possess at least three different substrate binding sites (i.e., a hydrophobic interaction site and a positively and a negatively charged binding site), the relative importance of accessibility of which might be variable in various Oatp isoforms, remains to be investigated in any detail. Nevertheless, our data support the conclusions that 1) OATP mediates the clearance of highly lipophilic organic cations in human liver, and 2) members of the *Oatp* gene family of membrane transporters are in general responsible for the previously reported increase in hepatic clearance with increasing lipophilicity of various organic compounds (Proost et al., 1997).

In conclusion, the present study provides definite evidence for transport of bulky type II organic cations by members of the *Oatp* gene family of membrane transporters. These results explain previous kinetic studies in the isolated perfused rat liver and in isolated hepatocytes and demonstrate that Oatp-mediated transport can account for the postulated multispecific organic cation transporter in rat and human livers (Steen et al., 1992; Meijer et al., 1997). Further work is required to define the structural features that are required to qualify organic compounds as Oatp substrates and to investigate the substrate-transporter interactions and the mechanism of transmembrane solute movement in more detail.

Table 1: Comparison of organic cation transport activities between the organic anion transporting polypeptides Oatp1, Oatp2, and OATP. *Xenopus laevis* oocytes were injected with 5 ng of Oatp1-cRNA, 5 ng of Oatp2-cRNA, and 2.5 ng of OATP-cRNA. After 3 days in culture, substrate uptakes were determined during 30 min at the indicated substrate concentrations in PBS (see *Experimental Procedures*). The standard substrates estrone-3-sulfate, digoxin and DHEAS were used as positive controls for adequate expression of the cRNA-injected oocytes. Data represent the mean ± S.D. of 11 to 15 oocyte uptake measurements. Level of significant difference from water controls is **p* < 0.001, (Mann-Whitney *U* test). ND, not determined.

	Uptake rate			
	Water	**Oatp1**	**Oatp2**	**OATP**
		fmol/oocyte·min		
Control substrate				
Estrone-3-sulfate (1 μM)	0.5 ± 0.1	16.6 ± 5.5*	ND	9.5 ± 3.5*
Digoxin (1 μM)	1.7 ± 0.2	ND	9.6 ± 2.1*	ND
DHEAS (1 μM)	0.3 ± 0.1	21.4 ± 5.3*	2.5 ± 0.5*	5.5 ± 1.6*
Type II Organic Cations				
N-methyl-quinine (2 μM)	8.0 ± 2.5	13.8 ± 2.6*	8.8 ± 1.9	71.8 ± 22.9*
N-methyl-quinidine (2 μM)	9.8 ± 3.0	9.1 ± 2.0	8.3 ± 1.6	67.8 ± 20.5*
APDA (2 μM)	4.8 ± 1.5	11.7 ± 3.5*	38.5 ± 12.9*	71.6 ± 30.6*
Rocuronium (14 μM)	67.4 ± 17.0	110.1 ± 26.7*	152.2 ± 32.5*	171.1 ± 32.7*
Type I Organic Cations				
TBuMA (2 μM)	14.3 ± 3.8	18.6 ± 3.4	11.2 ± 2.0	16.7 ± 3.3
APM (2 μM)	16.5 ± 5.4	14.1 ± 2.3	19.2 ± 4.0	24.2 ± 6.1
APQ (2 μM)	8.8 ± 2.0	10.9 ± 1.0	6.3 ± 1.0	9.9 ± 1.9
Choline (2 μM)	14.3 ± 3.2	18.6 ± 2.3	15.1 ± 5.7	11.2 ± 1.9

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